Hydrophobins.dia KCC/12-16-2004

File 5:Biosis Previews(R) 1969-2004/Dec W1

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| S1 | 211 | HYDROPHOBIN? |
| S2 | 5 | (FUSION()PROTEIN) AND S1 |
| S3 | 26 | TRICHODERMA AND (FUSION()PROTEIN) |
| S4 | 5 | S3 AND PURIF? |
| S5 | 1147 | AQUEOUS()TWO()PHASE |
| S6 | 6 | S3 AND S5 |
| S7 | 5 | S1 AND S5 |
| S8 | 2 | S7 NOT S2 |
| S9 | 2 | S7 NOT S4 |
| S10 | 0 | S9 NOT S8 |
| S11 | 3 | PARTITIONING()PEPTIDE? |

**? t s2/7/1-5**

2/7/1

DIALOG(R)File 5:Biosis Previews(R)

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0014892070 BIOSIS NO.: 200400262827

Large-scale separation and production of engineered proteins, designed for facilitated recovery in detergent-based aqueous two-phase extraction systems.

AUTHOR: Selber Klaus; Tjerneld Folke; Collen Anna; Hyytia Teppo; Nakari-Setala Tiina; Bailey Michael; Fagerstrom Richard; Kan John; van der Laan Joop; Penttila Merja; Kula Maria-Regina (Reprint)

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JOURNAL: Process Biochemistry 39 (7): p889-896 March 31, 2004 2004

MEDIUM: print

ISSN: 1359-5113 _(ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The feasibility and scalability of extraction in detergent-based aqueous two-phase systems for the separation of proteins from culture broth is demonstrated. At the same time the large-scale production of a %%%fusion%%% %%%protein%%% and the influence of cultivation scale on the efficiency of separation were investigated. An amphiphilic %%%fusion%%% %%%protein%%% EGIcore-HFBI was chosen, consisting of the catalytic core of the cellulase endoglucanase I and the small protein %%%hydrophobin%%% I, expressed homologously in Trichoderma reesei. Using the technical nonionic detergent Agrimul NRE 1205 the separation was successfully scaled up to 1200 1. No differences in yield or in partition coefficient were observed at 10 ml and 1200 1 scale. Changes in the fermentation temperature and scale, however, can influence the properties of the protein and thus alter partition coefficient and yield. The decreased separation efficiency appears to be correlated with changes in glycosylation at lower cultivation temperatures.

2/7/2

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0014426658 BIOSIS NO.: 200300383935

Intracellular processing and secretion of the fungal %%%hydrophobin%%% cryparin.

AUTHOR: Turina M (Reprint); Kazmierczak P; van Alfen N
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JOURNAL: Phytopathology 93 (6 Supplement): pS85 June 2003 2003
MEDIUM: print
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SPONSOR: American Phytopathological Society
ISSN: 0031-949X _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cryparin is an abundant class II fungal %%%hydrophobin%%% found in the cell walls of fruiting bodies of the fungus Cryphonectria parasitica. This protein is necessary for the eruption of the fungal fruiting body through the bark of infected trees. Large amounts of cryparin are secreted in liquid culture allowing its use in the study of vesicular protein secretion. The preprocryparin is processed by cleavage of the signal peptide and then the propeptide is cleaved by a Kex2-type endoprotease. The role of the Kex2-type processing on secretion of this protein was studied by site-specific mutagenesis of the Kex2 recognition site. Antibodies were raised against a His-tag cryparin %%%fusion%%% %%%protein%%% and used in Western blot analysis of subcellar fractions and culture fluid of C. parasitica. GFP fusion was also used to study the localization of cryparin. Results indicate that Kex2 processing is not necessary for secretion and that cryparin localizes within discrete bodies in the hyphae.

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0013940252 BIOSIS NO.: 200200533763

Surface adhesion of fusion proteins containing the %%%hydrophobins%%% HFBI and HFBII from Trichoderma reesei

AUTHOR: Linder Markus (Reprint); Szilvay Geza R; Nakari-Setala Tiina; Soderlund Hans; Penttila Merja

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JOURNAL: Protein Science 11 (9): p2257-2266 September, 2002 2002

MEDIUM: print

ISSN: 0961-8368

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: %%%Hydrophobins%%% are surface-active proteins produced by

filamentous fungi, where they seem to be ubiquitous. They have a variety of roles in fungal physiology related to surface phenomena, such as adhesion, formation of surface layers, and lowering of surface tension. %%%Hydrophobins%%% can be divided into two classes based on the hydropathy profile of their primary sequence. We have studied the adhesion behavior of two Trichoderma reesei class II %%%hydrophobins%%%, HFBI and HFBII, as isolated proteins and as fusion proteins. Both %%%hydrophobins%%% were produced as C-terminal fusions to the core of the hydrolytic enzyme endoglucanase I from the same organism. It was shown that as a fusion partner, HFBI causes the %%%fusion%%% %%%protein%%% to efficiently immobilize to hydrophobic surfaces, such as silanized glass and Teflon. The properties of the surface-bound protein were analyzed by the enzymatic activity of the endoglucanase domain, by surface plasmon resonance (Biacore), and by a quartz crystal microbalance. We found that the HFBI fusion forms a tightly bound, rigid surface layer on a hydrophobic support. The HFBI domain also causes the %%%fusion%%% %%%protein%%% to polymerize in solution, possibly to a decamer. Although isolated HFBII binds efficiently to surfaces, it does not cause immobilization as a fusion partner, nor does it cause polymerization of the %%%fusion%%% %%%protein%%% in solution. The findings give new information on how %%%hydrophobins%%% function and how they can be used to immobilize fusion proteins.

2/7/4

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0013666830 BIOSIS NO.: 200200260341

A novel two-step extraction method with detergent/polymer systems for primary recovery of the %%%fusion%%% %%%protein%%% endoglucanase I-%%%hydrophobin%%% I

AUTHOR: Collen Anna; Persson Josefine; Linder Markus; Nakari-Setala Tiina; Penttila Merja; Tjerneld Folke (Reprint); Sivars Ulf

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JOURNAL: Biochimica et Biophysica Acta 1569 (1-3): p139-150 15 January,

2002 2002
MEDIUM: print
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Extraction systems for hydrophobically tagged proteins have been developed based on phase separation in aqueous solutions of non-ionic detergents and polymers. The systems have earlier only been applied for separation of membrane proteins. Here, we examine the partitioning and purification of the amphiphilic %%%fusion%%% %%%protein%%% endoglucanase Icore-%%%hydrophobin%%% I (EGIcore-HFBI) from culture filtrate originating from a Trichoderma reesei fermentation. The micelle extraction system was formed by mixing the non-ionic detergent Triton X-114 or Triton X-100 with the hydroxypropyl starch polymer, Reppal PES100. The detergent/polymer aqueous two-phase systems resulted in both better separation characteristics and increased robustness compared to cloud point extraction in a Triton X-114/water system. Separation and robustness were characterized for the parameters: temperature, protein and salt additions. In the Triton X-114/Reppal PES100 detergent/polymer system EGIcore-HFBI strongly partitioned into the micelle-rich phase with a partition coefficient (K) of 15 and was separated from hydrophilic proteins, which preferably partitioned to the polymer phase. After the primary recovery step, EGIcore-HFBI was quantitatively back-extracted (KEGIcore-HFBI=150, yield=99%) into a water phase. In this second step, ethylene oxide-propylene oxide (EOPO) copolymers were added to the micelle-rich phase and temperature-induced phase separation at 55degreeC was performed. Total recovery of EGIcore-HFBI after the two separation steps was 90% with a volume reduction of six times. For thermolabile proteins, the back-extraction temperature could be decreased to room temperature by using a hydrophobically modified EOPO copolymer, with slightly lower yield. The addition of thermoseparating co-polymer is a novel approach to remove detergent and effectively releases the %%%fusion%%% %%%protein%%% EGIcore-HFBI into a water phase.

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DIALOG(R)File 5:Biosis Previews(R)

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0013566126 BIOSIS NO.: 200200159637

Extraction of endoglucanase I (Cel7B) fusion proteins from Trichoderma reesei culture filtrate in a poly(ethylene glycol)-phosphate aqueous two-phase system

AUTHOR: Collen Anna; Penttila Merja; Stalbrand Henrik; Tjerneld Folke; Veide Andres (Reprint)

AUTHOR ADDRESS: Department of Biotechnology, Royal Institute of Technology (KTH), Roslagstullsbacken 21, SE-10691, Stockholm, Sweden**Sweden

JOURNAL: Journal of Chromatography A 943 (1): p55-62 11 January, 2002 2002

MEDIUM: print

ISSN: 0021-9673

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Endoglucanases (EGI) (endo-1,4-beta-D-glucan-4-glucanohydrolase, EC 3.2.1.4, Cel7B) of Trichoderma reesei are industrially important enzymes. Thus, there is a great need for development of a primary recovery method suitable for large-scale utilization. In this study we present a concept applicable for large-scale purification of an EGI %%%fusion%%% %%%protein%%% by one-step extraction in a poly(ethylene glycol) PEG-sodium/potassium phosphate aqueous two-phase system. EGI is a two-module enzyme composed of an N-terminal catalytic module and a C-terminal cellulose binding module (CBM) separated by a glycosylated linker region. Partitioning of six different EGI constructs, containing the C-terminal extensions (WP)2, (WP)4 or the amphiphilic protein %%%hydrophobin%%% I (HFB) of T. reesei instead of the CBM were studied to evaluate if any of the fusions could improve the partition coefficient sufficiently to be suitable for large-scale production. All constructs showed improved partitioning in comparison to full length EGI. The (WP)4 extensions resulted in 26- to 60-fold improvement of partition coefficient. Consequently, a relative minor change in amino acid sequence on the two-module protein EGI improved the partition coefficient

significantly in the PEG 4000-sodium/potassium phosphate system. The addition of HFBI to EGI clearly enhanced the partition coefficient (K = 1.2) in comparison to full-length EGI (K = 0.035). Partitioning of the construct with (WP)4 fused to the catalytic module and a short sequence to the linker (EGIcore-P5(WP)4) resulted in the highest partition coefficient (K = 54) and a yield of 98% in the PEG phase. Gel electrophoresis showed that the construct with the (WP)4 tag attached after a penta-proline linker could be purified from the other bulk proteins by only a single-step separation in the PEG 4000-sodium/potassium phosphate system. This is a major improvement in comparison with the previously studied model (ethylene oxide-propylene oxide)-dextran system. Hence, this construct will be suitable for further optimization of the extraction of the enzyme in a PEG 4000-sodium/potassium phosphate system from culture filtrate.

**? t s4/7/1-5**

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0014892070 BIOSIS NO.: 200400262827

Large-scale separation and production of engineered proteins, designed for facilitated recovery in detergent-based aqueous two-phase extraction systems.

AUTHOR: Selber Klaus; Tjerneld Folke; Collen Anna; Hyytia Teppo; Nakari-Setala Tiina; Bailey Michael; Fagerstrom Richard; Kan John; van der Laan Joop; Penttila Merja; Kula Maria-Regina (Reprint)

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AUTHOR E-MAIL ADDRESS: mrk3372002@yahoo.de

JOURNAL: Process Biochemistry 39 (7): p889-896 March 31, 2004 2004

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0013666830 BIOSIS NO.: 200200260341

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AUTHOR: Collen Anna; Persson Josefine; Linder Markus; Nakari-Setala Tiina; Penttila Merja; Tjerneld Folke (Reprint); Sivars Ulf
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JOURNAL: Biochimica et Biophysica Acta 1569 (1-3): p139-150 15 January, 2002 2002
MEDIUM: print
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4/7/3

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0013566126 BIOSIS NO.: 200200159637

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0013199884 BIOSIS NO.: 200100371723
ACEII, a novel transcriptional activator involved in regulation of cellulase and xylanase genes of %%%Trichoderma%%% reesei
AUTHOR: Aro Nina (Reprint); Saloheimo Anu; Ilmen Marja; Penttila Merja
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JOURNAL: Journal of Biological Chemistry 276 (26): p24309-24314 June 29, 2001 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A novel yeast-based method to isolate transcriptional activators was applied to clone regulators binding to the cellulase promoter cbh1 of the filamentous fungus %%%Trichoderma%%% reesei (Hypocrea jecorina). This led to the isolation of the cellulase activator ace2 encoding for a protein belonging to the class of zinc binuclear cluster proteins found exclusively in fungi. The DNA-binding domain of ACEII was expressed as a glutathione S-transferase %%%fusion%%% %%%protein%%% in Escherichia coli, and ACEII was shown to bind in vitro to the 5'-GGCTAATAA site present in the cbh1 promoter. This site also contains the proposed binding sequence

of the xylanase activator XlnR of Aspergillus niger. Mutation of the GGC triplet abolished ACEII binding. The function of ACEII was studied by analyzing the effects of ace2 deletion in the hypercellulolytic T. reesei strain ALKO2221. Deletion of the ace2 gene led to lowered induction kinetics of mRNAs encoding the major cellulases cellobiohydrolases I and II and endoglucanases I and II and to 30-70% reduced cellulase activity when the fungus was grown on medium containing Solka floc cellulose. The expression level of the gene encoding xylanase was also affected. ace2 deletion led to lowered xyn2 expression in cellulose-induced cultivation. Cellulase induction by sophorose was not affected by ace2 deletion.

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0012330951 BIOSIS NO.: 200000049264
Heterologous expression and characterization of endoglucanase I (EGI) from %%%Trichoderma%%% viride HK-75
AUTHOR: Kwon II; Ekino Keisuke; Goto Masatoshi; Furukawa Kensuke (Reprint)
AUTHOR ADDRESS: Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kyushu University, Hakozaki, Fukuoka, 812-8581, Japan**Japan
JOURNAL: Bioscience Biotechnology and Biochemistry 63 (10): p1714-1720
Oct., 1999 1999
MEDIUM: print
ISSN: 0916-8451
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Endoglucanase I (EGI) secreted from %%%Trichoderma%%% viride HK-75 has a unique transglycosylation activity. The genomic and cDNA clones encoding EGI (egl1) of T. viride HK-75 were isolated and characterized. The coding region of egl1, composed of 1392 bp, was found to encode a polypeptide of 464 amino acids that has extensive similarity (93.8%) with EGI of T. reesei. Expression of the egl1 gene in E. coli as

a %%%fusion%%% %%%protein%%% (with N-terminal thioredoxin and C-terminal histidine tag) led to a large production of a nonglycosylated protein of 62.5 kDa. However, it formed an insoluble inclusion body. Upon denaturation with 8 M urea followed by dialysis and successive %%%purification%%%, the enzymatically active recombinant EGI (rEGI) was obtained at a level as high as 18.3 mg/l of 1,000 ml of culture. The rEGI had 67.8% activity for carboxymethyl cellulose (CMC), compared to native EGI (nEGI). The optimum pH and optimum temperature of rEGI were lower than those of nEGI by 0.5 and 5degreeC, respectively. The rEGI also had narrower CMCase ranges than nEGI in pH and temperature stabilities. However, the catalytic and transglycosylation abilities against cellotriose of rEGI were comparable to those of nEGI. These results suggest that the glycosylation is important for the stabilities of EGI but not critical for the essential enzymatic capacity.

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6/7/1

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0014892070 BIOSIS NO.: 200400262827

Large-scale separation and production of engineered proteins, designed for facilitated recovery in detergent-based %%%aqueous%%% %%%two%%%-%%%phase%%% extraction systems.

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ABSTRACT: The feasibility and scalability of extraction in detergent-based %%%aqueous%%% %%%two%%%-%%%phase%%% systems for the separation of proteins from culture broth is demonstrated. At the same time the large-scale production of a %%%fusion%%% %%%protein%%% and the influence of cultivation scale on the efficiency of separation were investigated. An amphiphilic %%%fusion%%% %%%protein%%% EGIcore-HFBI was chosen, consisting of the catalytic core of the cellulase endoglucanase I and the small protein hydrophobin I, expressed homologously in %%%Trichoderma%%% reesei. Using the technical nonionic detergent Agrimul NRE 1205 the separation was successfully scaled up to 1200 1. No differences in yield or in partition coefficient were observed at 10 ml and 1200 1 scale. Changes in the fermentation temperature and scale, however, can influence the properties of the protein and thus alter partition coefficient and yield. The decreased separation efficiency appears to be correlated with changes in glycosylation at lower cultivation temperatures.

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0013965089 BIOSIS NO.: 200200558600

Parameters influencing protein extraction for whole broths in detergent based %%%aqueous%%% %%%two%%%-%%%phase%%% systems

AUTHOR: Selber Klaus; Collen Anna; Hyytia Teppo; Penttila Merja; Tjerneld Folke; Kula Maria-Regina (Reprint)

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JOURNAL: Bioseparation 10 (4-5): p229-236 2001 2001

MEDIUM: print

ISSN: 0923-179X

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LANGUAGE: English

ABSTRACT: The parameters important for an optimisation of cloud point

extraction in technical scale were investigated using a genetically engineered %%%fusion%%% %%%protein%%% derived from endoglucanase I expressed in %%%Trichoderma%%% reesei and the nonionic polyoxyethylene Agrimul NRE 1205. The key parameters are temperature, detergent concentration, and additional salts. These parameters are interdependent, thus there is an optimum in the partition coefficient with respect to detergent concentration and a maximum for the partition coefficient and the yield with respect to temperature. These results were confirmed for the detergent C12E5 to demonstrate that these optima are due to the nature of polyoxyethylenes. Cloud point extraction was found to be only slightly affected by pH. In the case studied extraction of whole broth is favourable for a high yield and partition coefficient, since %%%fusion%%% %%%protein%%% adhering to the cells can be solubilized. However some loss of detergent which remains in the fungal biomass was observed.

6/7/3

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0013732421 BIOSIS NO.: 200200325932

Primary recovery of a genetically engineered %%%Trichoderma%%% reesei endoglucanase I (Cel 7B) %%%fusion%%% %%%protein%%% in cloud point extraction systems

AUTHOR: Collen Anna; Selber Klaus; Hyytia Teppo; Persson Josefine; Nakari-Setla Tiina; Bailey Michael; Fagerstrom Richard; Kula Maria-Regina ; Penttila Merja; Stalbrand Henrik (Reprint); Tjerneld Folke
AUTHOR ADDRESS: Department of Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, S-221 00, Lund, Sweden**Sweden

JOURNAL: Biotechnology and Bioengineering 78 (4): p385-394 May 20, 2002 2002
MEDIUM: print
ISSN: 0006-3592
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Here we present data to demonstrate how partitioning of a hydrophilic enzyme can be directed to the hydrophobic detergent-enriched phase of an %%%aqueous%%% %%%two%%%-%%%phase%%% system by addition of short stretches of amino acid residues to the protein molecule. The target enzyme was the industrially important endoglucanase I, EGI (endo-1,4-beta-D-glucan-4-glucanohydrolase, EC 3.2.1.4, Cel7B) of %%%Trichoderma%%% reesei. We investigated the partitioning of three EGI variants containing various C-terminal peptide extensions including Trp-Pro motifs of different lengths and localizations. Additionally, a recently developed system composed of the thermoseparating copolymer HM-EOPO was utilized to study the effects of fusion tags. The addition of peptides containing tryptophan residues enhanced the partitioning of EGI to the HM-EOPO-rich phase. The system composed of a nonionic detergent (Agrimul NRE1205) resulted in the highest partition coefficient (K=31) and yield (90%) with the construct EGIcore-P5(WP)4 containing (Trp-Pro)4 after a short linker stretch. A recombinant strain of T. reesei Rut-C30 for large-scale production was constructed in which the %%%fusion%%% %%%protein%%% EGIcore-P5(WP)4 containing (Trp-Pro)4 was expressed from the strong promoter of the cellulase gene cbh1. The %%%fusion%%% %%%protein%%% was successfully expressed and secreted from the fungus during shake-flask cultivations. Cultivation in a 28-L bioreactor however, revealed that the %%%fusion%%% %%%protein%%% is sensitive to proteases. Consequently, only low production levels were obtained in large-scale production trials.

6/7/4

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0013666830 BIOSIS NO.: 200200260341

A novel two-step extraction method with detergent/polymer systems for primary recovery of the %%%fusion%%% %%%protein%%% endoglucanase I-hydrophobin I

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ABSTRACT: Extraction systems for hydrophobically tagged proteins have been developed based on phase separation in aqueous solutions of non-ionic detergents and polymers. The systems have earlier only been applied for separation of membrane proteins. Here, we examine the partitioning and purification of the amphiphilic %%%fusion%%% %%%protein%%% endoglucanase Icore-hydrophobin I (EGIcore-HFBI) from culture filtrate originating from a %%%Trichoderma%%% reesei fermentation. The micelle extraction system was formed by mixing the non-ionic detergent Triton X-114 or Triton X-100 with the hydroxypropyl starch polymer, Reppal PES100. The detergent/polymer %%%aqueous%%% %%%two%%%-%%%phase%%% systems resulted in both better separation characteristics and increased robustness compared to cloud point extraction in a Triton X-114/water system. Separation and robustness were characterized for the parameters: temperature, protein and salt additions. In the Triton X-114/Reppal PES100 detergent/polymer system EGIcore-HFBI strongly partitioned into the micelle-rich phase with a partition coefficient (K) of 15 and was separated from hydrophilic proteins, which preferably partitioned to the polymer phase. After the primary recovery step, EGIcore-HFBI was quantitatively back-extracted (KEGIcore-HFBI=150, yield=99%) into a water phase. In this second step, ethylene oxide-propylene oxide (EOPO) copolymers were added to the micelle-rich phase and temperature-induced phase separation at 55degreeC was performed. Total recovery of EGIcore-HFBI after the two separation steps was 90% with a volume reduction of six times. For thermolabile proteins, the back-extraction temperature could be decreased to room temperature by using a hydrophobically modified EOPO copolymer, with slightly lower yield. The addition of thermoseparating co-polymer is a

novel approach to remove detergent and effectively releases the %%%fusion%%% %%%protein%%% EGIcore-HFBI into a water phase.

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Extraction of endoglucanase I (Cel7B) fusion proteins from %%%Trichoderma%%% reesei culture filtrate in a poly(ethylene glycol)-phosphate %%%aqueous%%% %%%two%%%-%%%phase%%% system

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ABSTRACT: Endoglucanases (EGI) (endo-1,4-beta-D-glucan-4-glucanohydrolase, EC 3.2.1.4, Cel7B) of %%%Trichoderma%%% reesei are industrially important enzymes. Thus, there is a great need for development of a primary recovery method suitable for large-scale utilization. In this study we present a concept applicable for large-scale purification of an EGI %%%fusion%%% %%%protein%%% by one-step extraction in a poly(ethylene glycol) PEG-sodium/potassium phosphate %%%aqueous%%% %%%two%%%-%%%phase%%% system. EGI is a two-module enzyme composed of an N-terminal catalytic module and a C-terminal cellulose binding module (CBM) separated by a glycosylated linker region. Partitioning of six different EGI constructs, containing the C-terminal extensions (WP)2, (WP)4 or the amphiphilic protein hydrophobin I (HFB) of T. reesei instead of the CBM were studied to evaluate if any of the fusions could improve the partition coefficient sufficiently to be suitable for large-scale

production. All constructs showed improved partitioning in comparison to full length EGI. The (WP)4 extensions resulted in 26- to 60-fold improvement of partition coefficient. Consequently, a relative minor change in amino acid sequence on the two-module protein EGI improved the partition coefficient significantly in the PEG 4000-sodium/potassium phosphate system. The addition of HFBI to EGI clearly enhanced the partition coefficient (K = 1.2) in comparison to full-length EGI (K = 0.035). Partitioning of the construct with (WP)4 fused to the catalytic module and a short sequence to the linker (EGIcore-P5(WP)4) resulted in the highest partition coefficient (K = 54) and a yield of 98% in the PEG phase. Gel electrophoresis showed that the construct with the (WP)4 tag attached after a penta-proline linker could be purified from the other bulk proteins by only a single-step separation in the PEG 4000-sodium/potassium phosphate system. This is a major improvement in comparison with the previously studied model (ethylene oxide-propylene oxide)-dextran system. Hence, this construct will be suitable for further optimization of the extraction of the enzyme in a PEG 4000-sodium/potassium phosphate system from culture filtrate.

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0012979393 BIOSIS NO.: 200100151232

Genetically engineered peptide fusions for improved protein partitioning in %%%aqueous%%% %%%two%%%-%%%phase%%% systems: Effect of fusion localization on endoglucanase I of %%%Trichoderma%%% reesei

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ABSTRACT: Genetic engineering has been used for fusion of the peptide tag, Trp-Pro-Trp-Pro, on a protein to study the effect on partitioning in %%%aqueous%%% %%%two%%%-%%%phase%%% systems. As target protein for the fusions the cellulase, endoglucanase I (endo-1,4-beta-D-glucan-4-glucanohydrolase, EC 3.2.1.4, EGI, Cel7B) of %%%Trichoderma%%% reesei was used. For the first time a glycosylated two-domain enzyme has been utilized for addition of peptide tags to change partitioning in %%%aqueous%%% %%%two%%%-%%%phase%%% systems. The aim was to find an optimal fusion localization for EGI. The peptide was (1) attached to the C-terminus end of the cellulose binding domain (CBD), (2) inserted in the glycosylated linker region, (3) added after a truncated form of EGI lacking the CBD and a small part of the linker. The different constructs were expressed in the filamentous fungus T. reesei under the gpdA promoter from Aspergillus nidulans. The expression levels were between 60 and 100 mg/l. The partitioning behavior of the fusion proteins was studied in an %%%aqueous%%% %%%two%%%-%%%phase%%% model system composed of the thermoseparating ethylene oxide (EO)-propylene oxide (PO) random copolymer EO-PO (50:50) (EO50PO50) and dextran. The Trp-Pro-Trp-Pro tag was found to direct the %%%fusion%%% %%%protein%%% to the top EO50PO50 phase. The partition coefficient of a %%%fusion%%% %%%protein%%% can be predicted with an empirical correlation based on independent contributions from partitioning of unmodified protein and peptide tag in this model system. The fusion position at the end of the CBD, with the spacer Pro-Gly, was shown to be optimal with respect to partitioning and tag efficiency factor (TEF) was 0.87, where a fully exposed tag would have a TEF of 1.0. Hence, this position can further be utilized for fusion with longer tags. For the other constructs the TEF was only 0.43 and 0.10, for the tag fused to the truncated EGI and in the linker region of the full length EGI, respectively.

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0013823126 BIOSIS NO.: 200200416637

Expression of a fungal %%%hydrophobin%%% in the Saccharomyces cerevisiae cell wall: Effect on cell surface properties and immobilization

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ABSTRACT: The aim of this work was to modify the cell surface properties of Saccharomyces cerevisiae by expression of the HFBI %%%hydrophobin%%% of the filamentous fungus Trichoderma reesei on the yeast cell surface. The second aim was to study the immobilization capacity of the modified cells. Fusion to the Flo1p flocculin was used to target the HFBI moiety to the cell wall. Determination of cell surface characteristics with contact angle and zeta potential measurements indicated that HFBI-producing cells are more apolar and slightly less negatively charged than the parent cells. Adsorption of the yeast cells to different commercial supports was studied. A twofold increase in the binding affinity of the %%%hydrophobin%%%-producing yeast to hydrophobic silicone-based materials was observed, while no improvement in the interaction with hydrophilic carriers could be seen compared to that of the parent cells. Hydrophobic interactions between the yeast cells and the support are suggested to play a major role in attachment. Also, a slight increase in the initial adsorption rate of the %%%hydrophobin%%% yeast was observed. Furthermore, due to the engineered cell surface, %%%hydrophobin%%%-producing yeast cells were efficiently separated in an

%%%aqueous%%% %%%two%%%-%%%phase%%% system by using a nonionic polyoxyethylene detergent, C12-18EO5.

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0013674119 BIOSIS NO.: 200200267630

The %%%hydrophobins%%% HFBI and HFBII from Trichoderma reesei showing efficient interactions with nonionic surfactants in %%%aqueous%%% %%%two%%%-%%%phase%%% systems

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ABSTRACT: Fungal %%%hydrophobins%%% are a group of surface active, self-assembling proteins. The filamentous fungus Trichoderma reesei produces two (class II) %%%hydrophobins%%%, HFBI and HFBII. We have studied how these water-soluble %%%hydrophobins%%% behave in two-phase systems using a series of nonionic surfactants with different characteristics. It was found that both %%%hydrophobins%%%, but especially HFBI, had a very high affinity for the surfactants. The highest partitioning coefficient, over 2500, was observed for HFBI with C11EO2. Reducing the disulfides in the protein resulted in a complete loss of affinity for the surfactant, which demonstrates that the interaction is dependent on the disulfide-stabilized conformation. The %%%hydrophobins%%% could be efficiently extracted back from the surfactant phase by addition of alcohols such as isobutanol. Effects of the type of surfactant, temperature, pH, and ionic strength were investigated. The use of this method for purifying the proteins from

crude fungal culture supernatants is demonstrated and implications of the protein-polymer interaction are discussed.

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Functional selection of phage displayed peptides for facilitated design of fusion tags improving aqueous two-phase partitioning of recombinant proteins

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ABSTRACT: Aqueous two-phase systems allow for the unequal distribution of proteins and other molecules in water-rich solutions containing phase separating polymers or surfactants. One approach to improve the partitioning properties of recombinant proteins is to produce the proteins as fused to certain peptide tags. However, the rational design of such tags has proven difficult since it involves a compromise between multivariate parameters such as partitioning properties, solvent accessibility and production/secretion efficiency. In this work, a novel approach for the identification of suitable peptide tag extensions has been investigated. Using the principles of selection, rather than design, peptide sequences contributing to an improved partitioning have been identified using phage display technology. A 40 million member phagemid library of random nona-peptides, displayed as fusion to the major coat protein pVIII of the filamentous phage M13, was employed in the selection

of top-phase partitioning phage particles in a PEG/sodium phosphate system. After multiple cycles of selection by %%%partitioning%%%, %%%peptides%%% with high frequencies of both tyrosine and proline residues were found to be over represented in selected clones. The identified peptide sequences, or derivatives thereof, were subsequently individually analyzed for their partitioning behavior as displayed on phage, as free synthetic peptides and as genetically fused to a recombinant model target protein. The results showed that novel peptide sequences capable of enhancing top-phase partitioning without interfering with protein production and secretion indeed could be identified for the aqueous two-phase system investigated.

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Peptides partitioning in an aqueous dextran-polyethylene glycol two-phase system

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ABSTRACT: Partitioning of glycine, lysine and aspartic acid and their oligopeptides in an aqueous dextran-polyethylene glycol two-phase system containing 0.15 M NaCl in 0.01 sodium phosphate buffer, pH 7.3 and 0.11 M sodium phosphate buffer, pH 7.3 was examined. Relative hydrophobicity of the amino acid residues and peptide bonds was estimated and expressed in equivalent numbers of methylene units. Analysis of a series of reversed

di- and tripeptides in terms of relative hydrophobicity showed that the additivity principle does hold for the hydrophobicity of short peptides. The relative hydrophobicity of peptides is affected by the ionic composition of aqueous media as well as by the type of amino acid residues forming peptide bonds in a given peptide sequence.

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Folding of amphipathic alpha-helices on membranes: Energetics of helix formation by melittin

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JOURNAL: Journal of Molecular Biology 285 (4): p1363-1369 Jan. 29, 1999

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ABSTRACT: Membranes have a potent ability to promote secondary structure formation in a wide range of membrane-active peptides, believed to be due to a reduction through hydrogen bonding of the energetic cost of %%%partitioning%%% %%%peptide%%% bonds. This process is of fundamental importance for understanding the mechanism of action of toxins and antimicrobial peptides and the stability of membrane proteins. A classic example of membrane-induced folding is the bee-venom peptide melittin that is largely unstructured when free in solution, but strongly adopts an amphipathic alpha-helical conformation when partitioned into membranes. We have determined the energetics of melittin helix formation through measurements of the partitioning free energies and the helicities of native melittin and of a diastereometric analog with four D-amino

acids (D4,L-melittin). Because D4,L-melittin has little secondary structure in either the free or bound forms, it serves as a model for the experimentally inaccessible unfolded bound form of native melittin, The partitioning of native melittin into large unilamellar phosphocholine vesicles is 5.0(+0.7) kcal mol-1 more favorable than the partitioning of D4,L-melittin (1 cal = 4.186 J). Differences in the circular dichroism spectra of the two forms of melittin indicate that bound native melittin is more helical than bound D4,L-melittin by about 12 residues. These findings disclose that the free energy reduction per residue accompanying the folding of melittin in membrane interfaces is about 0.4 kcal mol-1, consistent with the hypothesis that hydrogen bonding reduces the high cost of %%%partitioning%%% %%%peptide%%% bonds. A value of 0.6 kcal mol-1 per residue has been observed for beta-sheet formation by a hexapeptide model system. These two values provide a useful rule of thumb for estimating the energetic consequences of membrane-induced secondary structure formation.

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